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FACTORS AFFECTING THE TOXICITY OF THE ELEMENT INDIUM

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SUMMARY.—Hydrated indium oxide is 40 times more toxic than ionic indium, when expressed as lethality per quantity of metal injected.

Ionic indium is nephrotoxic, causing damage in the proximal portion of the proximal convoluted tubule. In this respect, it resembles the element mercury. At extremely high doses, ionic indium causes focal necrosis in the liver.

Hydrated indium oxide causes damage to those organs which contain phagocytic cells which clear the insoluble particles from the blood after i.v. injection. Actual focal necrosis was found in the liver, spleen and bone marrow. Damage was also found in the thymus and lymph nodes. At extremely high doses, damage was observed in the proximal convoluted tubules of the kidney.

Hydrated indium oxide caused extensive haemorrhage and marked thrombocytopenia. Fibrin thrombi were observed in the liver.

The increase in toxicity of indium resulting from phagocytosis of insoluble oxides by the reticuloendothelial system may represent a general mechanism by which the toxicity of certain heavy metals is increased.

INDIUM is a Group III-B heavy metal widely distributed in minute quantities in nature (Fassett and Irish, 1966). One of the principal commercial uses of indium is in surface protection of metals, particularly for prevention of corrosion of cadmium and lead alloys. Other commercial uses include its addition to dental alloys, graphite, glass to glass seals, motion picture screens, cathode oscillographs, transistors and infra-red detectors (Hodgman, 1963).

With its use as an industrially important metal has come the potential problem of toxicity. Of the 24 known isotopes of indium, only 2 are found in nature, indium-113 which is stable and comprises 4·33 per cent of naturally occurring indium, and indium-115 which is radioactive with a physical half life of 6×10^5 yr (95·67 per cent abundance) (Sunderman and Townley, 1960). The dusts, grindings and filings associated with milling or the spraying of various indium salts are potential routes for contamination of the biosystem with the element.

A number of studies on the toxicity of indium had been made prior to the present investigations (McCord, Meek, Harrold and Hauser, 1942; Downs, Scott and Steadman, 1959; Harrold, Meek, Whitman and McCord, 1943; Smith, Thomas, Black and Scott, 1957; Smith and Scott, 1957; Marrow, Gibb, Cloutier, Casarett and Scott, 1958). In 1942, McCord *et al.*, found that parenterally administered indium is one of the most toxic of elements. This finding was later confirmed by Downs *et al.* (1959). Toxicity data was reported on indium chloride (citrated) and indium sulphate in rabbits, rats, guinea-pigs, and man. The

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human studies were limited to skin tests. Indium chloride, administered to rats, rabbits and dogs as the citrate complex, had an acute lethal dose, ranging from 0·33–3·6 mg. indium per kg. body wt, with major damage to the kidneys (Downs *et al.*, 1959). Parenterally administered indium sesquioxide was far less toxic: single i.p. doses of 955 mg. indium per kg. body wt or greater were required to produce death of rats 9 days after injection. Indium sesquioxide was moderately toxic when administered i.v. to rabbits, 100 mg. indium per kg. body wt resulting in death 1–3½ months after injection, with major damage to the lungs (Downs *et al.*, 1959). With multiple i.v. injections of this chemical form of indium, doses as high as 424 mg. per kg. body wt did not result in death of rabbits in 35 days. When ingested by rats, indium sesquioxide was nontoxic: incorporation of as much as 8 per cent in the diet for 3 months had no effect on growth, mortality or tissue morphology. In contrast, indium chloride caused a slight growth depression when it reached 2·4 per cent of the diet. A marked depression of growth was observed when indium comprised 4 per cent of the diet over a 3 month period.

Silver discs containing indium and implanted s.c., i.m. and i.p. in animals induced foreign body responses (Harrold *et al.*, 1943). They studied the absorption of indium by foods stored in indium plated containers. Significant amounts of the element were extracted by acidic foods; indeed, all of the plated indium was apparently leached out into the contents of the cans.

Our interest in the toxicity of indium resulted from the use of indium as a diagnostic radiopharmaceutical (Stern, Goodwin, Scheffel, Wagner and Kramer, 1967; Stern, Goodwin, Wagner and Kramer, 1966). Carrier-free indium-113m is administered i.v. in clinical medicine in 2 chemical forms: ionic indium chloride at pH 3·0 and colloidal hydrated indium oxide at pH 7·4. The ionic agent is used to delineate the distribution of vascular pools within the body; the colloidal agent is used to study the reticuloendothelial system (RES) and the pulmonary circulation. When injected at pH 3·0 ionic indium is bound by the metal-binding protein, transferrin (Hosaine, McIntyre, Poulouse, Stern and Wagner, 1969). In aqueous solution at pH 4·0 or higher, ionic indium forms insoluble hydrated oxide (Moeller, 1941).

MATERIALS AND METHODS

A. Preparation of indium compounds.—The nuclides of indium used in this study were radioactive indium-114m (physical half-life 50 days), and reagent grade indium-115 (physical half-life 6×10^5 yr) which served as carrier. The indium-114m was obtained from Oak Ridge National Laboratory, and indium-115 was obtained from K and K Laboratories as anhydrous indium trichloride. A solution of indium-115 containing 10 mg. of trivalent indium per ml. served as a stock solution. The highest level of radioactivity administered to each animal did not exceed 1 microcurie for indium-114m. Such radiation doses would not be expected to produce short-term pathological effects and none were observed in the control animals.

The ionic indium chloride was prepared by using a technique modified from Stern and Goodwin (Wagner, 1968). The following reagents were added sequentially: Hydrochloric Acid (0·05N) 8·9 ml.; Indium Carrier (Indium-115) × ml.; Radioactive Indium (Indium 114m) 25·0 lambda; Sodium Chloride (120 mg./ml.) 0·5 ml.; Sodium Hydroxide (0·5N and 0·1N) adjust to pH 3·0. The final solution was sterilized at 123° for 20 min.

The colloidal hydrated indium oxide (RES agent) was prepared by using a technique modified from Goodwin, Stern and Wagner (1967). The following reagents were added sequentially: Hydrochloric Acid (0·05N) 8·0 ml.; Indium Carrier (Indium-115) × ml.; Radioactive Indium (Indium-114m) 25·0 lambda; Gelatin (10 per cent) 1·0 ml.; Sodium Chloride (120 mg./ml.) 0·5 ml.; Sodium Hydroxide (0·5 and 0·1N) adjust to pH 7·4. The final solution was sterilized at 123° for 20 min.

B. Equipment and Counting Techniques.—All samples were counted in a Packard, Series 410A, well scintillation counter with a 5 in. NaI (Tl) crystal. Sufficient counting time was used to keep statistical errors below 5 per cent.

The indium-114m samples were counted at constant geometry with a window of 100 KeV to infinity.

C. Lethality studies.—All compounds were injected into the tail vein of adult male (20–25 g.) HRA/IRC white mice (purchased from Hazelton Animal Farm, Burtonsville, Maryland) at a rate of 0·02 ml. per sec. The levels administered were expressed as the number of mg. indium per kg. body weight. Control mice were injected with the vehicle used for the ionic indium and the hydrated indium oxide; no harmful effects were noted.

Four groups of 10 mice each were used for the acute lethality (LD_{50}) studies. Each animal received a single dose of ionic indium or hydrated indium oxide. The LD_{50} at 4 days was calculated according to the method of Miller and Tainter (1944). If deaths did not occur by 4 days, the animals survived at least 70 days, when the observations were terminated.

The dosage levels administered throughout this study were: for ionic indium—tracer quantity (Indium-114m), LD_0 (7·5 mg. Indium/kg.), LD_{50} (12·5 mg. Indium/kg.), LD_{100} (16·5 mg. Indium/kg.); for hydrated Indium oxide—tracer quantity (Indium-114m), LD_0 (0·103 mg. Indium/kg.), LD_{50} (0·323 mg. Indium/kg.), LD_{100} (0·825 mg. Indium/kg.).

D. Plasma protein binding.—Starch gel electrophoresis was employed to study protein binding. Four groups of 2 mice each were injected with Indium-114m ionic medium. Each group received a different dosage level of indium. After 30 min., all mice were killed by decapitation. The cervical venous blood from each group was collected in heparinized tubes and the plasma was isolated after centrifugation. Two 5 lambda samples from each dosage level were electrophoresed on starch gel (pH 8·6 Veronal Buffer, 0·05 Ionic strength) and, after 16 hr, staining of the gel and measurement of radioactivity were carried out.

E. Tissue distribution of indium.—In order to attempt to relate toxicity to the biological distribution of ionic and colloidal indium, studies were carried out in 4 groups of 36 mice per group for each compound. The individual groups were injected with tracer, LD_0, LD_{50}, and LD_{100} dosage levels. At each of the following times after administration, 3 mice were killed: 1, 5, 10, 15, and 30 min.; 1, 3, and 6 hr; 1, 2, 3 and 4 days. At the time of death, the liver, spleen, lung, kidney, blood (7 per cent body wt), brain, adrenal, and thyroid were isolated and their content of radioactivity measured. The percentage dose per total organ, the percentage dose per g. of tissue, and the standard deviations of each were calculated.

F. Whole body retention and excretion.—To study the rate of elimination of each agent from the body, 4 groups of 10 mice were each used for whole body retention studies. The mice were injected with 4 dosage levels of either ionic or colloidal indium and their total body radioactivity was measured for up to 70 days after injection.

For the excretion studies, 9 mice were injected with 3 dosage levels of ionic indium; 9 others were injected with similar dosage levels of hydrated indium oxide. After injection each mouse was placed in a metabolic cage and urine and faeces were collected at various time intervals up to 30 days after injection. The radioactivity of urine and faeces was measured in a gamma scintillation counter. The urine and faeces results were expressed as a percentage of injected dose excreted as a function of time. The whole body activity immediately after injection was taken as the 100 per cent dose.

G. Pathological studies.—Three groups of 8 mice each were used in pathological studies. The LD_0, LD_{50}, and LD_{100} dosage levels of both agents were injected and 2 animals were killed daily for 4 days. The following organs were removed from each animal: liver, kidney, spleen, lung, femur, thymus and lymph nodes. All organs were fixed in Lillies Neutral Buffered solution with 4 per cent formalin and subsequently embedded in paraffin. They were sectioned and stained with haematoxylin and eosin, and then analysed by consultants from comparative pathology (DVM).

H. Haematological studies.—Three groups of 10 mice each were injected with 3 dosage levels of hydrated indium oxide. On the day before injection and on the days following, a white blood cell count, platelet count, and differential count were obtained. Blood was obtained from the ocular vein.

I. Data processing and statistical analysis.—The calculations involved in analysing the results of the various experiments were mathematically simple but became very time consuming when the number of observations were large. For this reason, and to avoid trivial arithmetic errors, a Fortran IV program was written to process the raw data on an IBM 360 computer.

The standard statistical tests used here require no special comment and include the following: standard error of the mean; significance between means; student "t" test; chi square test; probit analysis.

RESULTS

A. Lethality of Indium Compounds

1. *Ionic indium*

The dosage levels and their respective mortalities are shown in Table I. No deaths were observed after 4 days. The LD_{50} 4 days after i.v. injection was calculated to be 12·5 mg. $\pm$ 0·58 (1 S.D.) per kg. for the ionic indium. The slope of the probit line was 14·82 $\pm$ 2·76 as illustrated in Fig. 1.

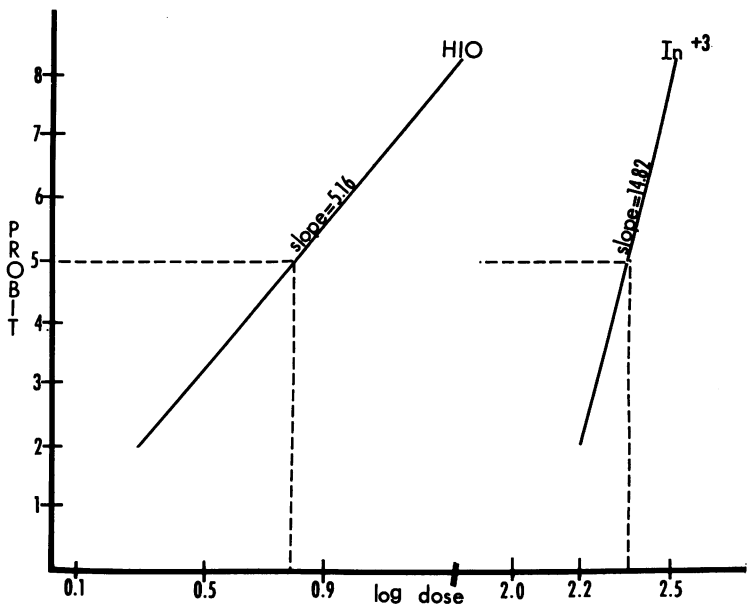


FIG 1.—Probit analysis of ionic indium and hydrated indium oxide toxicity data.

Lethal amounts of ionic indium had the following effects: inactivity of the animals and hind leg paralysis one day after injection; rapid and spasmodic respiration, weight loss and abnormal pelt after 2 days; at 3 days the mice became totally inactive and died by day 4.

2. *Hydrated indium oxide*

The dosage levels of hydrated indium oxide administered along with their respective mortality rates are listed in Table II. The LD_{50} 4 days after injection was calculated to be 0·323 $\pm$ 0·063 mg. of indium per kg. The resulting slope of the probit line was 5·16 $\pm$ 1·45 as illustrated in Fig. 1.

After administration of lethal amounts of hydrated indium oxide, the mice lost weight, became inactive, and developed hind leg paralysis after 2 days. They then developed muscle tremors and had clonic convulsions. Prior to death on day 3, the mice bled from the mouth, nose, ears, and intestines.

The LD_0 and LD_{100} dosage levels used throughout this study were taken as amounts of ionic indium or hydrated indium oxide that killed none or all of the animals respectively as shown in Tables I and II.

TABLE I.—*Ionic Indium Chloride LD_{50} 4 Day Study*

Group	Amount of Indium Ion administered (mg./kg.)	Number dead at 4 days
1	7·5	0/10
2	9·8	2/10
3	12·6	4/10
4	16·5	10/10

TABLE II.—*Hydrated Indium Oxide LD_{50} 4 Day Study*

Group	Amount of Indium Ion Administered (mg./kg.)	Number dead at 4 days
1	0·103	0/10
2	0·207	3/10
3	0·413	7/10
4	0·825	10/10

B. Biological Behaviour of Indium Related to Chemical Form

1. *Plasma protein binding of ionic indium*

The results of starch gel electrophoresis are illustrated in Fig. 2. The individual protein bands consist of γ-, β-, and α-globulins and albumin. The mouse has 2 transferrins (Watkins, Tee, Wang and Tarlow, 1966); on the basis of their different electrophoretic mobilities, one is called fast transferrin (f-trans.) and the other slow transferin (s-trans.).

The tracer dosage level of ionic indium (0·02 μg. of indium per 20 g. mouse) became bound to both transferrins, with small amounts being detected bound to α-globulin and albumin. The same results were obtained with the LD_0 and LD_{50} dosage levels, except that approximately 10 per cent of the dose was bound to transferrin. More remained at the origin and more was bound by other plasma proteins. With the LD_{100} dosage level, this observation was even more striking.

2. *Tissue distribution of indium*

a. Ionic indium.—The initial tissue distribution and subsequent translocation for tracer, LD_0, LD_{50}, and LD_{100} dosage levels of ionic and colloidal indium are illustrated in Tables III and IV.

After i.v. injection, the blood level of ionic indium fell until less than 1 per cent of the administered dose remained in this compartment after 3 days. This pattern was observed with all dosage levels, although the LD_0 and LD_{50} dosage levels were cleared more rapidly than either the tracer or the LD_{100} doses. Concomitant with its clearance from the blood, the activity in the kidneys and liver increased initially and then fell. On a per g. basis, the kidneys were the principal site of uptake of ionic indium at all dosage levels.

The subsequent fate of the indium after the initial accumulation in the kidneys decreased as the dosage level increased. With the LD_{100} dose, over 30 per cent of the dose per g. was in the kidneys after 4 days, compared to less than 20 per

TABLE III.—*Issue Distribution of Ionic Indium*

Tissue	Time	Tracer		LD_0		LD_{50}		LD_{100}	
		per organ m±SD	per g. m±SD	per organ m±SD	per g. m±SD	per organ m±SD	per g. m±SD	per organ m±SD	per g. m±SD
Blood	5 min.	75·67 ±8·04	36·98 ±5·17	69·10 ±8·81	33·55 ±4·39	83·41 ±1·68	40·98 ±3·10	84·08 ±5·16	38·56 ±2·50
	30 min.	42·32 ±6·24	23·51 ±2·25	45·31 ±10·60	24·61 ±4·92	43·40 ±12·47	29·76 ±7·24	47·89 ±4·25	28·91 ±3·87
	1 hr.	35·18 ±0·62	20·23 ±1·62	23·63 ±9·42	14·09 ±4·30	36·00 ±8·40	22·86 ±2·70	40·81 ±11·54	19·96 ±4·07
	3 hr.	21·73 ±0·29	11·17 ±0·26	13·12 ±6·46	7·40 ±5·67	22·53 ±4·21	11·95 ±3·08	22·07 ±1·76	13·41 ±3·26
	1 day	4·36 ±1·98	2·17 ±0·95	2·92 ±1·96	1·48 ±1·07	1·73 ±0·62	0·94 ±0·38	0·19 ±0·04	0·09 ±0·02
	3 days	0·42	0·41	—	—	—	—	Dead	Dead
Liver	5 min.	27·78 ±4·61	15·75 ±1·46	16·10 ±0·65	9·28 ±0·59	15·96 ±2·45	8·39 ±0·83	9·52 ±0·82	5·96 ±1·31
	30 min.	22·47 ±1·63	14·34 ±0·09	15·24 ±0·95	10·01 ±1·26	21·43 ±1·06	11·87 ±1·05	10·56 ±1·04	8·10 ±0·08
	1 hr.	26·78 ±4·25	17·54 ±3·66	19·88 ±7·47	11·43 ±4·17	15·85 ±0·58	12·17 ±1·81	10·82 ±0·46	6·86 ±1·17
	3 hr.	23·72 ±0·36	16·45 ±1·24	18·76 ±1·13	12·78 ±1·17	30·82 ±10·03	18·47 ±3·67	15·19 ±2·49	10·55 ±3·66
	1 day	17·99 ±5·83	9·92 ±1·77	12·78 ±2·09	8·33 ±2·43	12·20 ±1·00	8·56 ±1·26	12·75 ±0·87	7·36 ±0·32
	3 days	11·41 ±1·70	8·01 ±1·23	10·93 ±0·73	8·00 ±3·03	10·35 ±0·52	7·05 ±0·05	9·94 ±4·78	7·21 ±0·78
Kidneys	5 min.	15·26 ±5·44	41·59 ±15·21	3·92 ±0·53	11·24 ±0·95	5·25 ±1·83	12·36 ±2·55	5·25 ±2·14	10·75 ±2·06
	30 min.	10·41 ±0·78	32·63 ±6·40	7·41 ±3·25	18·78 ±7·59	8·02 ±1·72	23·12 ±1·54	11·35 ±2·29	29·71 ±2·73
	1 hr.	6·95 ±0·81	21·22 ±0·05	5·31 ±1·07	14·63 ±3·25	22·52 ±6·66	41·68 ±10·29	14·47 ±6·98	33·95 ±10·63
	3 hr.	9·16 ±0·12	22·09 ±0·66	17·23 ±3·94	55·64 ±3·39	17·55 ±1·96	40·38 ±5·16	20·60 ±1·08	53·28 ±6·70
	1 day	10·28 ±0·03	22·58 ±0·28	10·73 ±5·02	24·87 ±10·72	18·84 ±0·85	44·88 ±2·65	15·17 ±0·38	26·91 ±2·28
	3 days	8·48 ±2·90	20·35 ±6·05	6·89 ±1·40	16·94 ±4·58	7·01 ±3·14	17·83 ±8·26	20·86 ±1·06	33·14 ±2·42
Spleen	5 min.	0·73 ±0·33	8·92 ±2·59	0·25 ±0·05	1·91 ±0·68	0·48 ±1·14	3·67 ±1·13	0·42 ±0·19	3·44 ±0·66
	30 min.	0·91 ±0·09	10·47 ±1·49	0·73 ±0·06	4·85 ±0·49	0·51 ±0·23	4·72 ±1·56	0·50 ±0·05	5·30 ±0·92
	1 hr.	1·06 ±0·01	9·95 ±2·68	0·99 ±0·09	8·90 ±0·26	0·46 ±0·14	4·10 ±1·14	0·68 ±0·12	5·61 ±1·87
	3 hr	0·81 ±0·14	6·59 ±0·55	0·45 ±0·05	3·89 ±0·98	0·88 ±0·08	4·94 ±1·44	0·76 ±0·09	7·63 ±5·91
	1 day	0·96 ±0·30	6·74 ±0·78	0·78 ±0·19	5·26 ±0·76	0·76 ±0·23	7·07 ±4·56	1·19 ±0·22	9·92 ±0·37
	3 days	0·62 ±0·26	6·43 ±0·35	0·22 ±0·02	3·01 ±0·56	0·52 ±0·12	3·65 ±1·02	0·92 ±0·01	6·37 ±0·40

cent for the other dosage levels. Slight increases in activity in bone (femur) were observed with the LD_0 and LD_{50} doses. The significance of this is unknown.

b. hydrated indium oxide.—Almost immediately after injection, all dosage levels of hydrated indium oxide were cleared rapidly from the blood and accumulated in the liver and spleen. Other organs which concentrated detectable levels were

TABLE III.—*continued*

Tissue	Time	Tracer per organ m±SD	Tracer per g. m±SD	LD_0 per organ m±SD	LD_0 per g. m±SD	LD_{50} per organ m±SD	LD_{50} per g. m±SD	LD_{100}—W— per organ m±SD	LD_{100}—W— per g. m±SD
Skeleton (femur)	5 min.	—	12·82 ±1·59	—	2·84 ±0·27	—	1·92 ±0·54	—	2·06 ±0·32
	30 min.	—	9·98 ±1·39	—	4·29 ±1·69	—	6·30 ±4·13	—	6·98 ±1·38
	1 hr	—	7·41 ±1·51	—	1·98 ±1·04	—	8·32 ±3·17	—	9·27 ±2·26
	3 hr	—	6·57 ±0·08	—	7·92 ±0·56	—	8·99 ±2·47	—	10·87 ±1·92
	1 day	—	10·62 ±1·19	—	13·15 ±7·76	—	9·82 ±1·78	—	13·46 ±1·45
	3 days	—	11·87 ±2·39	—	17·48 ±7·78	—	20·23 ±7·55	—	15·35 ±0·50
Muscle	5 min.	—	4·25 ±1·86	—	0·89 ±0·05	—	0·98 ±0·26	—	0·70 ±0·07
	30 min.	—	8·27 ±4·73	—	0·96 ±0·03	—	0·86 ±0·22	—	1·65 ±0·58
	1 hr.	—	2·78 ±0·08	—	0·90 ±0·02	—	0·67 ±0·18	—	1·12 ±0·25
	3 hr		1·83 ±0·66	—	0·94 ±0·01	—	0·38 ±0·09	—	0·77 ±0·34
	1 days	—	1·88 ±0·24	—	0·92 ±0·46	—	0·41 ±0·21	—	0·20 ±0·03
	3 days	—	1·24 ±0·01	—	3·03 ±0·14	—	0·45 ±0·05	—	0·40 ±0·05
Lung	5 min.	6·57 ±2·19	34·22 ±8·97	6·27 ±3·01	10·21 ±5·32	0·07 ±0·01	1·05 ±0·74	0·42 ±0·12	16·21 ±4·31
	30 min.	4·20 ±1·76	15·41 ±8·32	1·34 ×0·32	10·04 ±4·32	3·10 ±1·24	13·21 ±6·41	0·31 ±0·09	12·12 ±4·96
	1 hr	2·86 ±1·01	9·52 ±5·62	0·73 ±0·21	3·63 ±0·96	3·24 ±1·14	13·02 ±6·24	1·84 ±0·94	9·02 ±3·21
	3 hr.	2·14 ±0·78	8·21 ±3·21	0·08 ±0·02	3·01 ±0·76	1·52 ±0·83	3·21 ±1·42	1·50 ±0·74	8·51 ±2·61
	1 days	1·02 ±0·86	4·54 ±3·56	0·04 ±0·02	2·04 ±0·78	0·04 ±0·01	2·00 ±0·92	0·03 ±0·01	2·81 ±0·65
	3 days	0·75 ±0·09	2·31 ±0·76	0·02 ±0·01	0·08 ±0·03	0·01 ±0·01	1·02 ±0·04	0·03 ±0·01	2·01 ±0·77

the kidneys, bone (femur), muscle and lung. On a per g. basis, the liver accumulated the highest amount.

After the initial uptake of hydrated indium oxide by the liver and spleen the fate of indium was related to the dose (Table IV). With the tracer dose, the activity stayed in the liver for up to 4 days; with the other dosage levels, the activity fell progressively with corresponding slight increases in the femur, kidneys, spleen, and to a lesser extent, in the muscles.

3. *Whole body retention and excretion*

a. Whole body retention.—The loss of ionic indium or hydrated indium oxide from the body of the mouse can be expressed as a fractional retention (R), which is equal to the sum of the two exponential terms as follows:

$$R = R_1^0 \, e^{-\text{lambda } t} + R_2^0 \, e^{-\text{lambda } t}$$

TABLE IV.—*Tissue Distribution of Hydrated Indium Oxide*

Tissue	Time	Tracer		LD_0		LD_{50}		LD_{100}	
		per organ m±SD	per g. m±SD	per organ m±SD	pre g. m±SD	per organ m±SD	per g. m±SD	per organ m±SD	per g. m±SD
Blood	5 min.	1·35 ±0·14	0·74 ±0·19	7·41 ±0·82	5·83 ±0·82	2·94 ±0·97	2·01 ±0·94	22·76 ±5·55	12·68 ±3·80
	30 min.	0·81 ±0·40	0·48 ±0·24	6·26 ±2·93	9·07 ±6·77	1·39 ±0·33	0·77 ±0·18	3·52 ±1·12	2·06 ±0·64
	1 hr	0·75 ±0·09	0·47 ±0·05	4·21 ±2·21	2·49 ±1·38	0·99 ±0·84	0·59 ±0·48	1·21 ±0·78	0·67 ±0·38
	3 hr	0·66 ±0·17	0·37 ±0·05	1·44 ±0·02	0·81 ±0·11	1·20 ±0·22	0·85 ±0·24	1·51 ±0·91	0·86 ±0·56
	1 days	0·30 ±0·05	0·19 ±0·07	1·33 ±0·79	0·92 ±0·51	1·29 ±0·09	0·77 ±0·01	1·26 ±0·10	0·69 ±0·07
	3 days	0·20 ±0·03	0·12 ±0·07	0·96 ±0·32	0·61 ±0·19	1·17 ±0·13	0·72 ±0·02	Dead	Dead
Liver	5 min.	86·37 ±1·62	53·60 ±8·10	61·47 ±4·49	45·55 ±3·80	77·75 ±19·24	88·25 ±19·68	60·20 ±4·48	40·73 ±5·96
	30 min.	87·49 ±0·28	54·23 ±2·44	67·51 ±4·64	54·78 ±6·09	76·94 ±1·88	53·22 ±3·79	77·51 ±4·00	63·54 ±12·81
	1 hr	82·51 ±6·02	69·89 ±4·59	74·80 ±2·10	48·67 ±6·29	72·19 ±5·86	48·22 ±8·36	70·09 ±6·85	50·77 ±11·70
	3 hr	78·97 ±10·32	60·35 ±1·70	69·14 ±2·27	48·29 ±5·76	79·38 ±21·87	63·00 ±15·65	77·05 ±8·83	54·37 ±4·96
	1 day	80·76 ±4·32	64·72 ±4·72	65·82 ±13·09	43·52 ±4·81	69·97 ±3·55	53·57 ±0·63	65·45 ±1·16	39·71 ±1·32
	3 days	80·12 ±6·12	64·52 ±8·31	50·25 ±1·73	27·28 ±3·38	51·03 ±2·12	37·50 ±5·80	50·10 ±12·15	39·83 ±8·72
Kidneys	5 min.	0·55 ±0·12	1·22 ±1·00	0·81 ±0·09	2·87 ±0·36	0·77 ±0·45	2·97 ±2·32	0·87 ±0·37	2·05 ±0·73
	30 min.	0·43 ±0·09	1·24 ±0·05	0·49 ±0·28	1·77 ±1·14	0·39 ±0·10	1·11 ±0·12	0·32 ±0·06	1·33 ±0·56
	1 hr	0·23 ±0·01	1·14 ±0·09	0·49 ±0·33	1·64 ±1·27	0·30 ±0·11	0·91 ±0·46	0·38 ±0·18	1·12 ±0·65
	3 hr	0·36 ±0·01	1·31 ±0·05	0·52 ±0·11	1·51 ±0·41	0·25 ±0·01	0·85 ±0·01	0·45 ±0·03	1·58 ±0·55
	1 day	0·83 ±0·31	0·61 ±0·31	1·54 ±0·76	5·31 ±3·38	2·01 ±0·03	7·01 ±0·08	1·66 ±0·34	5·17 ±0·03
	3 days	0·55 ±0·03	0·06 ±0·01	1·47 ±0·01	3·91 ±0·37	2·33 ±0·62	7·30 ±3·17	5·65 ±3·29	14·89 ±2·64
Spleen	5 min.	1·53 ±0·12	14·62 ±4·88	1·00 ±0·38	13·72 ±0·50	1·48 ±0·64	23·40 ±8·86	1·63 ±0·05	15·02 ±1·55
	30 min.	1·83 ±0·37	20·86 ±7·55	1·90 ±0·28	17·27 ±0·29	1·45 ±0·14	11·66 ±1·69	1·66 ±0·48	25·96 ±8·69
	1 hr.	1·66 ±1·00	31·68 ±11·04	1·81 ±0·27	17·02 ±5·69	1·06 ±0·13	8·46 ±2·87	1·49 ±0·48	13·39 ±6·93
	3 hr	1·79 ±0·63	21·96 ±3·93	1·69 ±0·11	13·03 ±0·75	1·39 ±0·05	9·65 ±0·18	2·17 ±0·69	18·50 ±0·94
	1 day	1·50 ±1·07	18·21 ±6·31	1·51 ±0·40	28·14 ±4·71	1·63 ±0·69	22·60 ±4·96	2·49 ±0·23	16·65 ±1·35
	3 days	1·40 ±1·20	16·31 ±7·24	1·32 ±0·96	20·21 ±3·61	1·60 ±0·92	20·34 ±5·21	4·62 ±1·18	27·21 ±7·23

Where:

R_1^0 = fractional retention of the fast component at some original time;
R_2^0 = fractional retention of the slow component at some original time;
lambda = biological decay constant for particular component, equal to $0{\cdot}693/T$ $1/2_b$ (T $1/2_b$ being biological half-life of component).

TABLE IV—*continued*

Tissue	Time	Tracer per organ m±SD	Tracer per g. m±SD	LD_0 per organ m±SD	LD_0 per g. m±SD	LD_{50} per organ m±SD	LD_{50} per g. m±SD	LD_{100} per organ m±SD	LD_{100} per g. m±SD
Skeleton (femur)	5 min.	—	0·51 ±0·02	—	5·51 ±2·36	—	3·64 ±1·57	—	2·14 ±1·22
	30 min.	—	0·31 ±0·07	—	3·95 ±0·25	—	2·77 ±1·04	—	4·93 ±1·56
	1 hr	—	1·03 ±0·34	—	3·99 ±0·22	—	2·82 ±1·85	—	4·74 ±0·07
	3 hr	—	0·37 ±0·07	—	2·18 ±0·96	—	2·74 ±0·94	—	3·26 ±1·43
	1 day	—	0·72 ±0·43	—	5·83 ±1·96	—	3·56 ±1·42	—	6·54 ±2·63
	3 days	—	0·80 ±0·41	—	5·72 ±0·21	—	5·53 ±0·93	—	7·10 ±0·49
Muscle	5 min.	—	0·11 ±0·03	—	0·46 ±0·06	—	0·26 ±0·21	—	0·57 ±0·12
	30 min.	—	0·28 ±0·06	—	0·34 ±0·26	—	0·42 ±0·78	—	1·08 ±0·84
	1 hr	—	1·39 ±0·68	—	0·15 ±0·11	—	0·58 ±0·18	—	0·73 ±0·23
	3 hr	—	0·03 ±0·01	—	0·01 ±0·01	—	0·49 ±0·12	—	0·65 ±0·01
	1 day	—	0·04 ±0·01	—	5·26 ±2·33	—	4·70 ±0·11	—	5·45 ±0·37
	3 days	—	0·50 ±0·04	—	5·15 ±0·07	—	6·24 ±0·15	—	7·27 ±0·12
Lung	5 min.	0·32 ±0·07	1·32 ±0·31	1·21 ±0·31	7·21 ±1·62	1·02 ±0·08	4·16 ±1·79	2·31 ±0·87	9·23 ±3·21
	30 min.	0·31 ±0·04	1·47 ±0·91	0·41 ±0·09	2·33 ±0·98	0·43 ±0·02	1·83 ±0·87	0·94 ±0·06	5·31 ±1·67
	1 hr	0·25 ±0·09	1·72 ±0·62	0·43 ±0·08	2·04 ±1·02	0·38 ±0·01	1·62 ±0·76	0·82 ±0·09	3·36 ±1·02
	3 hr	0·31 ±0·06	1·38 ±0·54	0·34 ±0·03	1·73 ±1·07	0·36 ±0·03	1·03 ±0·71	0·41 ±0·02	2·55 ±0·97
	1 day	0·23 ±0·09	0·94 ±0·16	0·23 ±0·02	1·40 ±0·86	0·91 ±0·06	1·72 ±0·62	0·49 ±0·03	2·86 ±1·2
	3 days	0·17 ±0·04	0·52 ±0·09	0·21 ±0·02	1·02 ±0·91	0·23 ±0·02	1·31 ±0·17	0·81 ±0·03	4·37 ±1·34

A summary of the retention data is given in Table V, which lists the fractional retention and biological half-life of the ionic indium and the hydrated indium oxide.

An initial fast component and a second slower component was observed for all dosage levels of both compounds, except the LD_{100} doses. With the LD_{100} dose,

TABLE V.—*Fractional Retention and Biological Half-life for all Components of Ionic Indium and Hydrated Indium Oxide*

Dose	Ionic indium: Fast (per cent)	Ionic indium: Fast $T\ 1/2_b$	Ionic indium: Slow (per cent)	Ionic indium: Slow $T\ 1/2_b$	Hydrated indium oxide: Fast (per cent)	Hydrated indium oxide: Fast $T\ 1/2_b$	Hydrated indium oxide: Slow (per cent)	Hydrated indium oxide: Slow $T\ 1/2_b$
Tracer	31	1·9 d.	69	69·0 d.	18·0	2·0 d.	82·0	73·8 d.
LD_0	50	2·1 d.	50	62·0 d.	24·5	2·0 d.	75·5	61·9 d.
LD_{50}	52	2·1 d.	48	74·5 d.	28·0	2·0 d.	72·0	71·0 d.
LD_{100}	60	retained at 4 days			100·0	retained at 4 days		

at the time of death at 4 days, approximately 60 per cent of the ionic indium was still in the body. In the case of hydrated indium oxide, approximately all the injected dose was retained at the time of death.

The fast components accounted for about half of the dose for all ionic indium dosage levels, except the tracer dose. The same figure for hydrated indium oxide was approximately 25 per cent for the LD_0 and LD_{50} dosage levels and 18 per cent for the tracer level. The biological half-life of the fast component ranged from 1·9–2·1 days for both compounds. The biological half-life of the slower component was similar for the ionic and particulate indium. This slower component for ionic indium ranged between 69·0–74·5 days. For hydrated indium oxide, the slower component ranged from 61·9–73·8 days.

There is no significant difference ($P > 0{\cdot}05$) between the biological half-lives for both compounds at similar dosage levels. However, there is a significant difference ($P < 0{\cdot}05$) between the percentages of fractional retention for fast and slow components for both compounds. Thus, the hydrated indium oxide particles were retained within the body to a greater degree than the ionic indium.

TABLE VI.—*Urine and Faeces Excretion Patterns for Sublethal and Lethal Dosage Levels of Ionic and Colloidal Indium*

	Ionic indium						Hydraged indium oxide					
	Per cent of injected dose excreted						Per cent of injected dose excreted					
	LD_0		LD_{50}		LD_{100}		LD_0		LD_{50}		LD_{100}	
Days	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine
0–1	6·73	28·11	6·23	10·72	5·42	33·24	7·61	2·53	8·72	3·31	1·34	0·76
1–2	6·66	3·94	5·61	12·23	1·14	0·12	5·28	0·82	9·10	3·62	0·22	0·15
2–3	4·72	4·23	4·21	2·11	0·74	0·11	4·64	0·91	2·41	0·81	—	—
3–6	7·08	5·43	3·01	8·21	—	—	8·32	1·29	9·45	0·92	—	—
6–8	1·05	2·89	2·03	3·41	—	—	6·08	0·92	5·21	0·62	—	—
9–10	1·34	2·47	1·06	2·34	—	—	3·42	0·92	2·15	0·75	—	—
10–13	2·41	1·05	2·12	2·23	—	—	4·56	0·23	3·21	0·15	—	—
13–16	1·76	2·46	2·24	2·05	—	—	4·03	0·36	5·11	0·32	—	—
16–20	1·34	1·11	1·56	2·34	—	—	3·13	1·05	4·12	1·34	—	—
20–23	0·57	0·11	1·01	0·98	—	—	2·20	0·27	3·21	1·01	—	—
23–30	0·92	0·66	0·89	0·88	—	—	3·84	0·38	3·15	0·97	—	—
Total	34·58	52·46	29·97	47·50	7·30	33·47	53·11	9·68	55·84	13·82	1·56	0·91

EXPLANATION OF PLATES

FIG. 2.—Starch gel electrophoresis of plasma after the i.v. injection of 114 m. Indium labelled ionic indium. The percentage of dose in plasma per sample versus sample number is illustrated relative to the individual protein bands on the stained starch gel. (CF=tracer). a=γ-globulin; b=slow transferrin; c=fast transferrin; d=α-globulin; e=albumin.

FIG. 3.—Kidney LD_{50} dosage level of ionic indium at 3 days, showing acute tubular necrosis. × 220

FIG. 4.—Liver, LD_{100} dose of ionic indium at 3 days—focal necrosis, × 355.

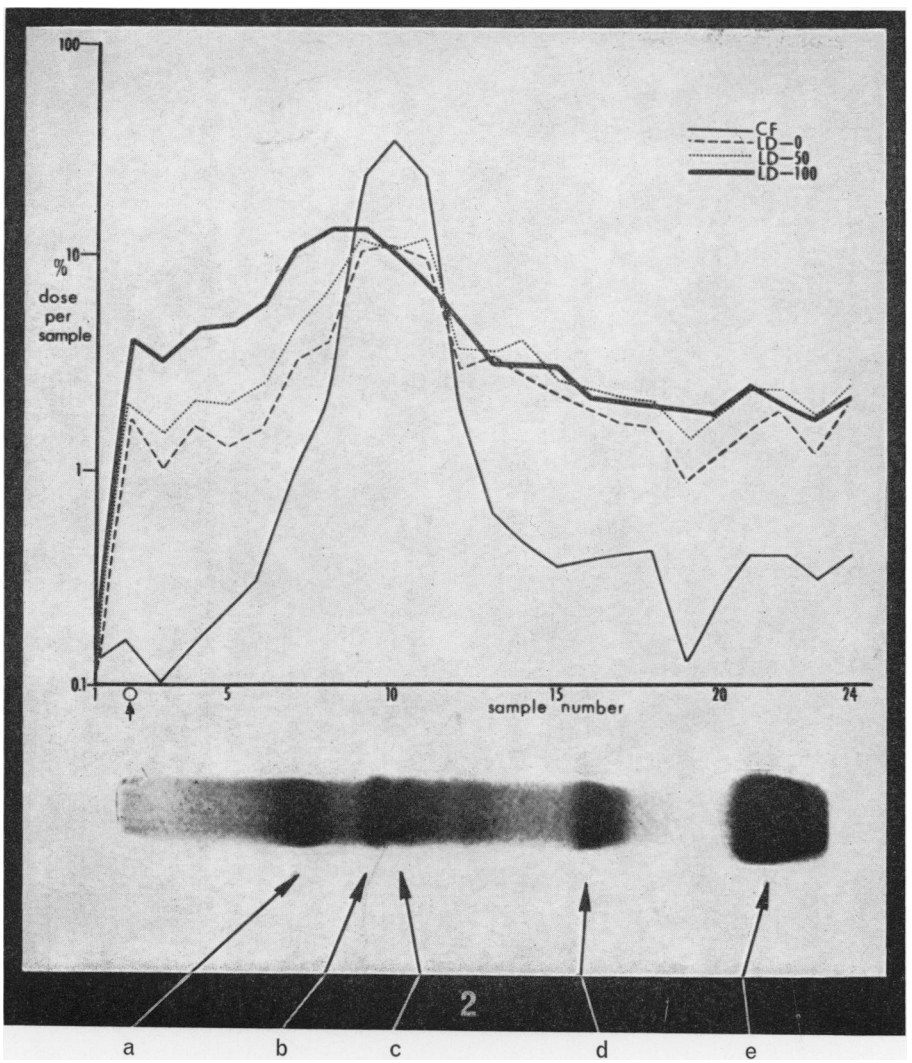
FIG. 5.—Liver LD_{50} dosage level of hydrated indium oxide at 3 days showing focal necrosis. × 115.

FIG. 6.—Macrophage loaded with hydrated indium oxide particles. × 900.

FIG. 7.—Spleen LD_{50} dosage level of hydrated indium oxide at 3 days. Lymphocytic depression. × 100.

FIG. 8.—Kidney, LD_{100} dosage level of hydrated indium oxide at 3 days showing acute tubular necrosis. × 135.

FIG. 9.—Bone marrow, LD_{50} dosage level of hydrated indium oxide at 3 days showing hypocellularity and focal necrosis. × 270.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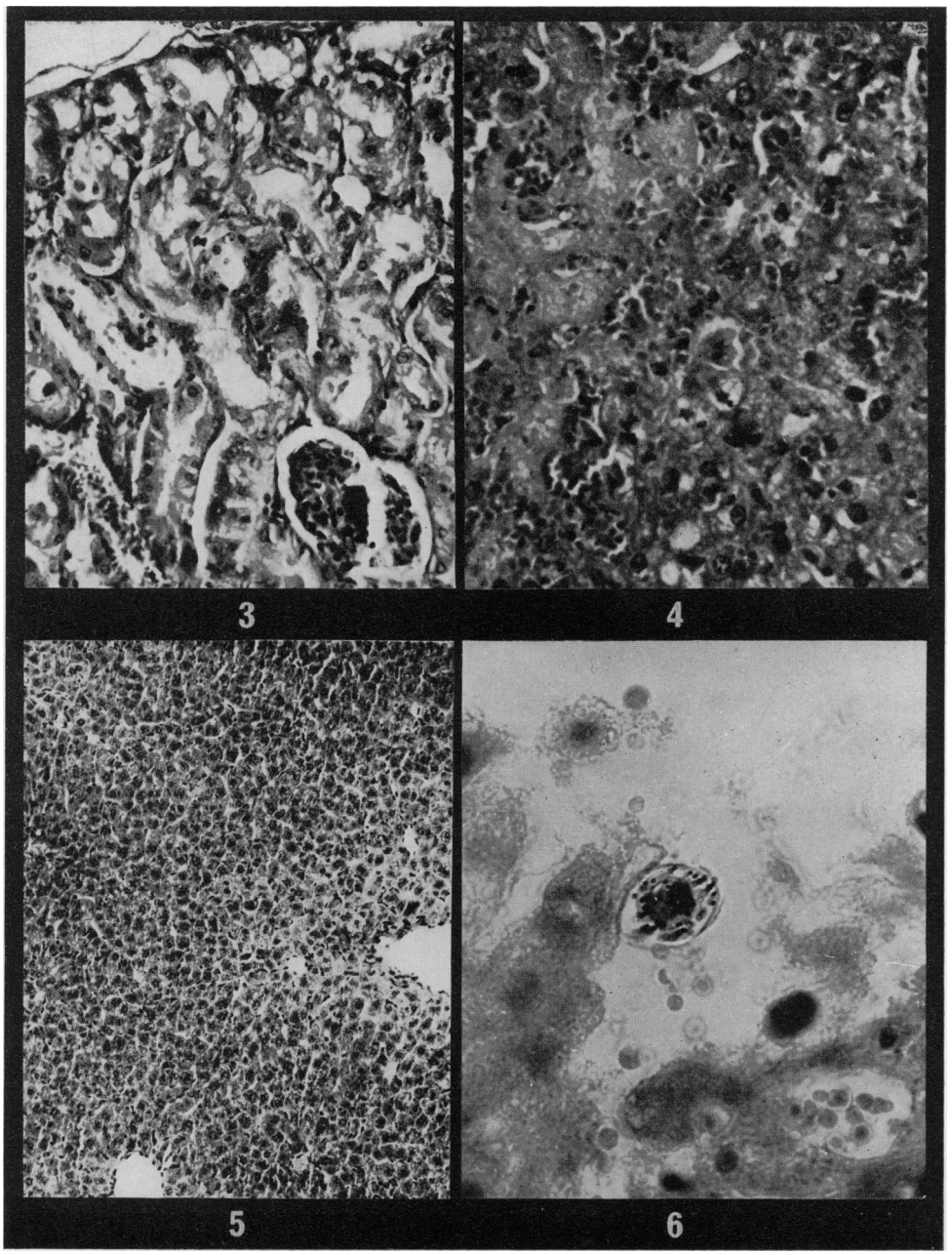

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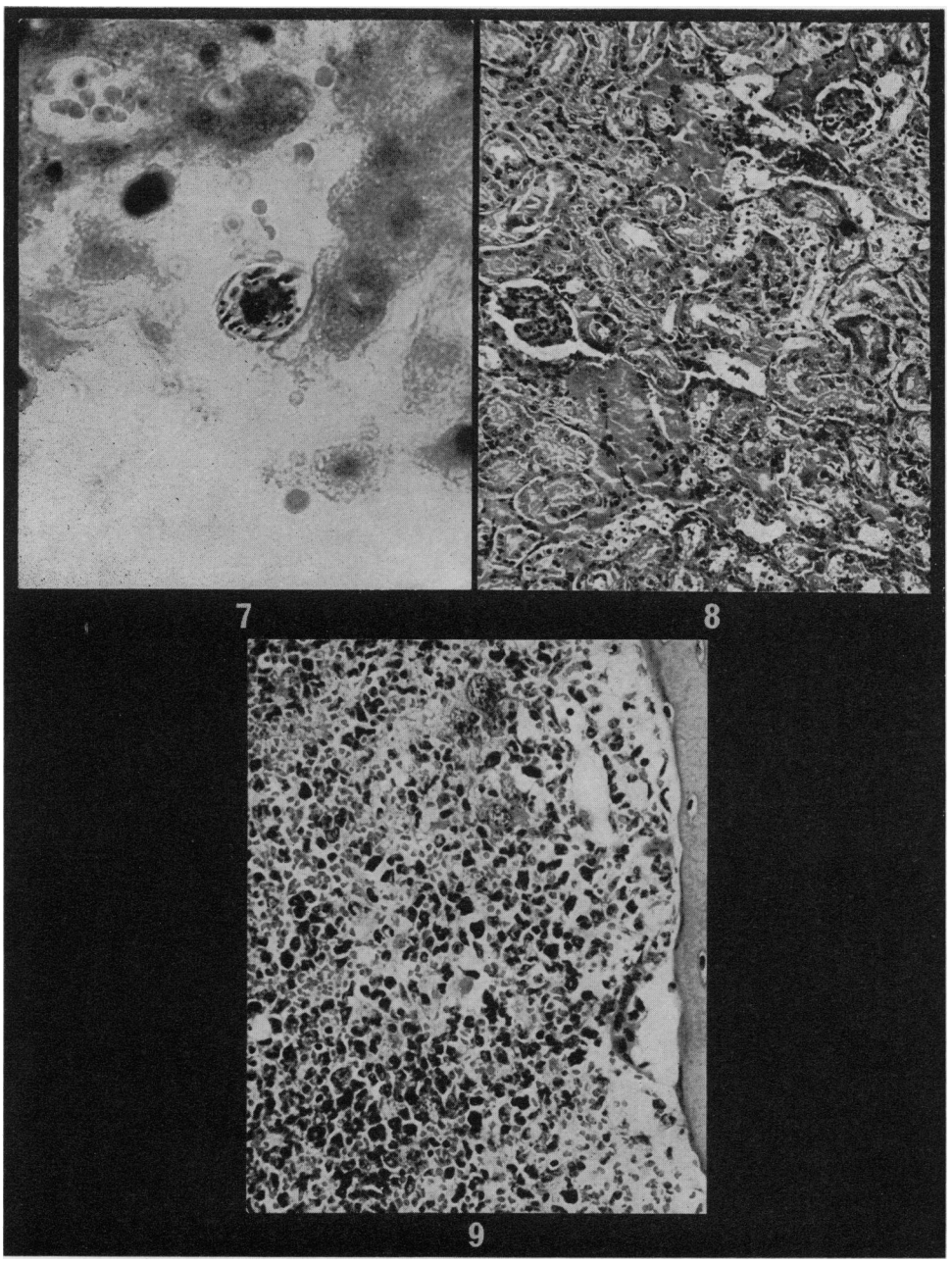

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b. Excretion.—The urinary and fecal excretion data for LD_0, LD_{50} and LD_{100} dosage levels of ionic and colloidal indium are summarized in Table VI. The ionic indium was excreted mainly in the faeces.

C. Pathology

1. *Ionic indium*

a. Kidney.—Ionic indium produced extensive damage to the kidney, with little damage to other organs. The major pathological findings in the kidney are as follows:

(1) LD_0 dosage level: The kidneys appeared normal and were used as controls.

(2) LD_{50} dosage level: Slight changes were observed, such as tubular epithelial swelling, occasional pyknotic nuclei, and eosinophilia of the proximal tubules, all occurring by the second day after injection. After 3 days some signs of regeneration were noted, including increased size of tubular cells and the presence of mitotic figures (Fig. 3).

(3) LD_{100} dosage level: The cells lining the convoluted tubules showed extensive necrosis as early as 1 day after injection. The proximal portion of the proximal convoluted tubule was affected most; in fact, most of the convoluted tubule was not affected. The necrotic changes included loss of cell margins, pyknosis, disintegration of nuclei, cytoplasmic eosinophilia, and loss of cytoplasmic structure. The remainder of the tubules and the glomeruli were not morphologically altered. Just before death, the necrotic tubules consisted of a structureless mass of eosinophilic material in which an occasional pyknotic nucleus was seen.

b. Liver.—In the liver were multiple small areas of necrosis in most of the mice injected with the LD_{50} and LD_{100} dosage levels. These lesions involved only parenchymal cells, with no changes in the Kupffer cells or the biliary system. Fig. 4 shows the focal necrosis produced after a LD_{100} dosage level of ionic indium.

2. *Hydrated indium oxide*

a. Liver.—No damage was found with the LD_0 dosage level of hydrated indium oxide. However, the LD_{50} (Fig. 5) and LD_{100} dosage levels produced extensive damage. The LD_{50} dosage level produced diffuse mild inflamation. The LD_{100} dosage level produced loss of glycogen, fatty changes, and moderate acute inflammatory infiltrates. Discrete amorphous, faintly eosinophilic intravascular masses of material, or aggregates which may have been fibrin thrombi were observed. The biliary system was unaffected.

The pathology was more marked in the LD_{100} group than in the LD_{50} group. All lesions described above were found in the LD_{100} mice.

Fig. 6 is a macrophage containing indium particles. No pathological changes were seen in the Kupffer cells.

b. Spleen.—The primary visible pathological changes produced in the spleen after hydrated indium oxide administration was a progression of lymphocytic depletion with increasing dosage level ($LD_{100} > LD_{50}$). In addition, other changes included extramedullary hematopoesis and indistinct splenic architecture, especially with the LD_{100} dosage level. The RES cells were normal in appearance. Fig. 7 shows the lymphocytic depression 3 days after a LD_{50} dosage level of HIO.

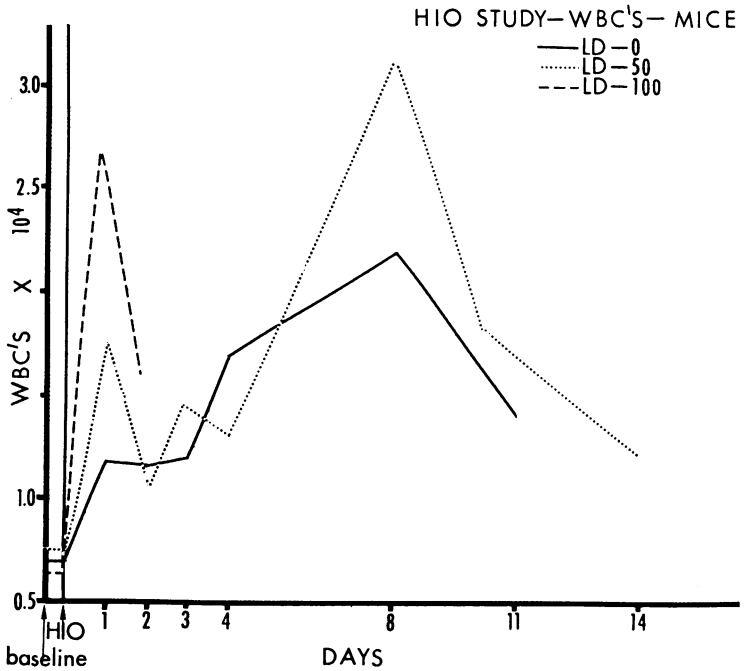

FIG. 10.—Effect of LD_0, LD_{50}, and LD_{100} dosage levels of hydrated indium oxide on the circulating white blood cells. Baseline values were made prior to H10 administration and observations were continued daily up to 14 days after injection.

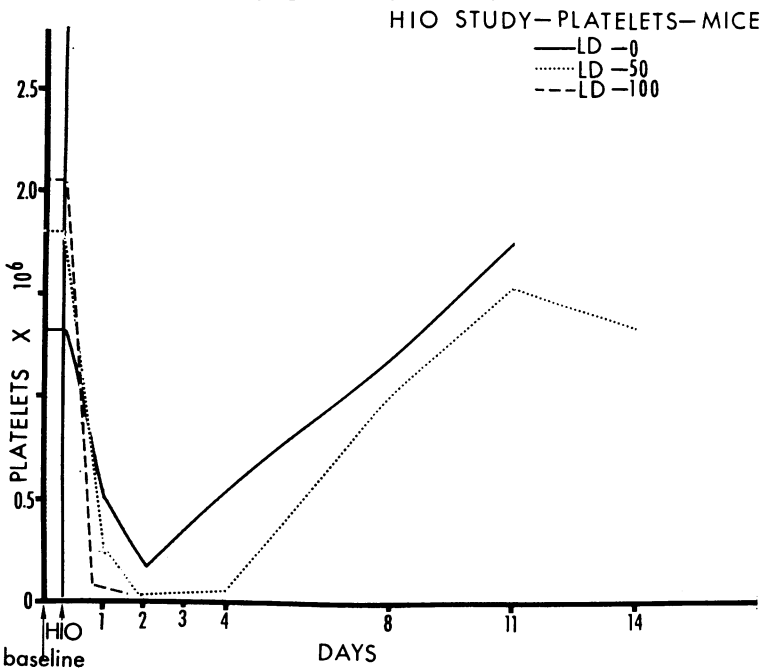

FIG. 11.—Effect of LD_0, LD_{50} and LD_{100} dosage levels of hydrated indium oxide on the circulating platelets. Baseline values were made prior to H10 administration and observations were continued daily up to 14 days after injection.

The HIO also produced extensive lymphopenia in the lymph nodes and the thymus.

c. Kidney.—The LD_0 and LD_{50} dosage levels produced no noticeable damage, but the LD_{100} dosage level produced acute tubular necrosis confined to the proximal portion of the proximal convoluted tubules, in a manner similar to the ionic indium (Fig. 8).

d. Other.—The large intestine was very bloody upon gross inspection, but histologically there seemed to be no damage. In the bone marrow, higher dosage levels of HIO produced extensive hypocellularity with many areas of focal necrosis (Fig. 9).

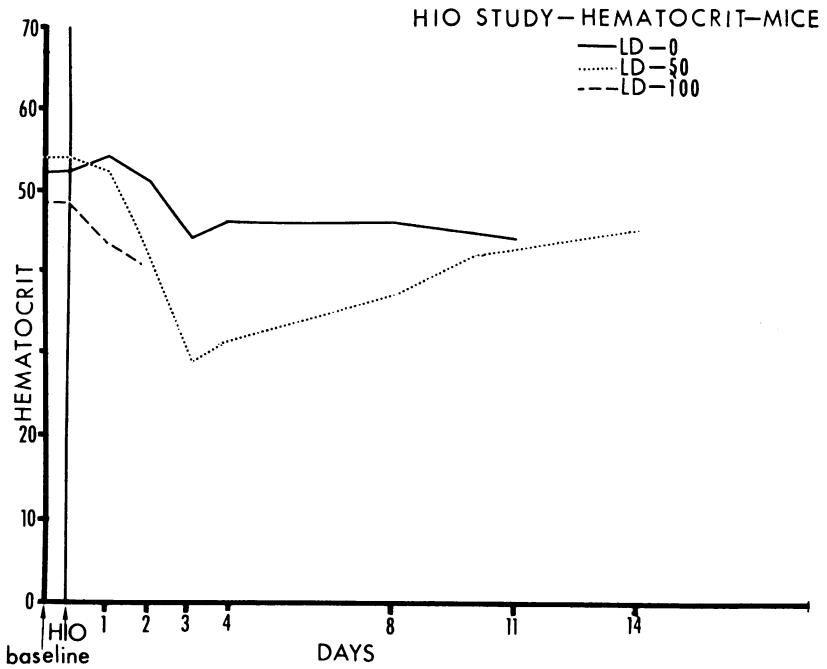

FIG. 12.—Effect of LD_0, LD_{50} and LD_{100} dosage levels of hydrated indium oxide on the haematocrit. Baseline values were made prior to H10 administration and observations were continued daily up to 14 days after injection.

3. *Damage to blood cells*

The haemorrhaging observed in the hydrated indium oxide treated mice prompted an investigation of the haematological effects of several dosage levels of this compound.

The LD_0 dosage level of hydrated indium oxide produced an almost immediate reduction in circulating platelets which persisted up to 2 days after injection. The platelet count returned to baseline values after 8 days. The circulating white blood cells (WBC) increased slightly up to 4 days after injection, and between 4–8 days, thereafter declining to near baseline values. The haematocrit changed slightly, reaching a low 3 days after injection and increased slightly thereafter, never quite reaching the previous baseline value (up to 14 days).

The LD_{50} dosage level produced a more rapid reduction in circulating platelets than in the LD_0 mice. This reduction persisted for up to 4 days, and rose almost to baseline values after 11 days. The haematocrit dropped up to 3 days after injection, and returned to near normal values after 14 days.

The LD_{100} injected mice did not survive for more than 2 days after injection. The most striking occurrence was an almost immediate drop in platelet count 18 hr after injection, which persisted until the time of death after 42 hr. There was no significant change in the haematocrit. Figs 10, 11, and 12 show the WBC, platelet and haemocrit results respectively.

The differential changed only slightly for all dosage levels of hydrated indium oxide.

Downs *et al.* (1959) reported on haematological changes in rats after an acute i.v. dose (LD_{50}) of indium chloride. The major changes included moderate alterations in haemoglobin, haematocrit and differential values. There was no observed change in platelet count.

DISCUSSION

Since the discovery of the process of phagocytosis by Metchinkoff (1883), emphasis has been placed on its beneficial effects to the organism: removal of effete cells from the body, defence against microbial infection, and removal of foreign material of various types from the circulating blood. The present experiments lead to the hypothesis that perhaps at times the phagocytic function of the RES may result in deleterious effects. In studies of the related toxicity of two chemical forms of the heavy metal, indium, it was found that the particulate form which is rapidly taken up by the cells of the RES is 40 times more toxic on a weight basis than the ionic form which is primarily bound to plasma proteins. While it is possible that the toxicity of the particulate form would have been even greater were it not for the phagocytic function of the RES, it is also possible that the process of phagocytosis, by localizing the material in high concentrations in the liver and other RES organs, enhanced the toxicity of the element.

Since indium has chemical properties similar to many heavy metals, *e.g.* highly insoluble at the pH of body fluids, perhaps what is true for indium may be true for other heavy metals as well. We believe that it is unlikely that the element indium is unique in this regard, and that perhaps a generalization can be made based on subsequent experiments.

At the pH of body fluids (7·4) ,hydrated indium oxide was rapidly cleared from the blood by phagocytic cells of the reticuloendothelial system. Ionic indium, injected at pH 3·0, became protein bound after injection, and only after the dosage was increased to high levels did aggregation occur. Kyker and Rafter (1966) reported previously that intravenously administered rare earths produced insoluble hydroxides, intermediate hydroxylated products, and metal proteinates in the blood. These colloids were cleared from the circulatory system as foreign particles by the RES, a fate similar to our findings with the indium aggregates.

The pathological changes produced by ionic indium were directly related to dosage level. As the dose increased, injury to the convoluted tubules of the kidney became more severe. Although the tracer and LD_0 dosage levels did not produce detectable microscopic damage to the kidneys, it is possible that the

acidity of the vehicle played some role in making the proximal tubule more susceptible to the effects of the indium. The toxic action of inorganic ionic indium on the kidney is probably the cause of death, and, in this respect, it resembles inorganic mercury (Harvey, 1965). Both metals exert their effects on the tubular epithelium, but renal lesions caused by mercury are also confined to the glomeruli (Sollman and Schreiber, 1936). Bickers, Bresler and Weinberger (1960) reported on the inhibition of the enzyme succinic dehydrogenase by mercury, and concluded that the metal's toxic action was related to inhibition of this -SH enzyme in the rat kidney tubule. Therefore, the toxic action of indium may also be related to enzyme inhibition, although future studies would have to be done to actually determine its principal site of action.

The LD_{50} for indium chloride in rats, rabbits, and dogs is cited in the literature to be 0·33–3·6 mg. of indium per kg. body wt (Harrold *et al.*, 1943). In mice, we found an LD_{50} value of 12·6 mg. of indium per kg. body wt. Previous studies were concerned with indium chelated with citrate at a pH of approximately 5·0. In the present study the indium was ionic, not chelated, and at a pH of 3·0. The difference in lethalities could also be due to species variation.

The lethality of the hydrated indium oxide had not been investigated previously. The addition of a stabilizing agent (gelatin) to insoluble indium kept it from forming a gelatinous precipitate, and resulted in the formation of colloidal particles. After i.v. injection, the resulting particles were rapidly cleared from the blood of the RES rather than being trapped by capillaries of the lungs (Goodwin *et al.*, 1967). Non-stabilized preparations of insoluble indium sesquioxide (Harrold *et al.*, 1943), were less toxic to rabbits than the stabilized hydrated oxide that was more readily phagocytized by the RES.

It is well established that phagocytosis leads to the dissolution of engulfed particles, such as micro-organisms, effete or injured cells, and microemboli, after their clearance from the blood (Heller, 1960). Inorganic particulate material, such as carbon and metallic colloids, and denatured or aggregated proteins are also cleared by the RES (Benacerrof and Delafresnay, 1957). Thus, hydrated indium oxide, a metallic colloid, was readily cleared from the blood, accumulated in the cells of the RES, and resulted in severe damage to hepatic parenchymal cells and lymphoid tissue of the spleen, lymph nodes and thymus. The damage caused to the thymus by the hydrated indium oxide requires special comment. Clark (1964), has shown that the epithelial cells of this organ are connected in a manner that creates a barrier between the blood capillaries and the interstices of the epithelial network where lymphocytes are produced. Clark (1964) has shown that this barrier is not complete since particulate matter can penetrate it. Nevertheless, the damage to the thymus may be related to a non-particulate form of the indium.

Before death, the mice bled from the ears, nose and mouth. At autopsy, signs of extensive haemorrhaging were found in the large intestine and liver, and the latter contained quantities of fibrin thrombi. This, coupled with the drastic thrombocytopenia suggested the animals died from bleeding and hepatotoxicity. The cause of the platelet drop is not known, but may be related to the extensive bleeding, intravascular clotting and/or direct action by the indium. Liver damage may have contributed to the haemorrhaging. Thus, after the injection of hydrated indium oxide, focal necrosis and haemorrhage resulted, which probably lead to systemic shock and blood vessel damage causing death.

The faeces was the main route of excretion for hydrated indium oxide. We

do not as yet know whether this is the result of passage through the biliary system or through the intestinal wall.

The chemical reactivity for insoluble hydrated indium oxide in a biological system might be expected to be less than for ionic indium since an insoluble chemical often has low reactivity. Thus, it was surprising to us that hydrated indium oxide was so much more toxic than the ionic form. In this regard, indium is similar to silicon dioxide, a compound which is quite insoluble, but is capable of producing toxic effects in biological systems, such as the lung (Sodeman and Sodeman, 1967).

Thus, we can extend our hypothesis on the mechanism of toxicity of hydrated indium oxide as follows: the insoluble colloid is accumulated in high concentrations in the RES by virtue of its physical nature; subsequent metabolism leads to toxicity related to its elemental chemical nature. How might an unreactive and inert colloid become " reactive " after phagocytosis of its insoluble oxide? Pinninger and Huett (1966), reported that after the administration of several colloidal iron compounds, the iron was initially picked up by the phagocytic cells of the liver and eventually found its way into the parenchymal cells of the liver 24–48 hr after injection. It may be that indium also passed into hepatic parenchymal cells after dissolution within the phagocyte. This is consistent with our observation that liver parenchymal damage first appeared 1–2 days after injection.

Phagocytosis usually results in the release of potent enzymes from lysosomes, which effectively destroy or alter engulfed material. In 1956, Sprick demonstrated that phagocytic cells have acidic vacuoles within their cytoplasm (Weissman, 1965). Since hydrated indium oxide is soluble in acidic solutions (Moeller, 1941), it seems reasonable that acid dissolution may be the mechanism by which unreactive hydrated indium oxide became reactive. After phagocytosis, the metalic colloid became solubilized in the acidic environment created in the vacuole by the enzymes. This activated form of indium (HIO–Ac), now present in high concentrations, may then exert its toxic effect on the parenchymal cells of those organs with large numbers of phagocytic cells. It may also have an effect on cells, such as platelets, as they pass through the RES.

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